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Use of Polymerase Chain Reaction to Provide Prognostic Information on Human Cytomegalovirus Disease After Liver Transplantation

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Sixty-four consecutive liver transplant patients receiving 76 organs have been monitored for human cytomegalovirus (HCMV) in blood and urine posttransplantation using a polymerase chain reaction (PCR) assay that amplifies a 149 base pair fragment of the glycoprotein B gene. Six hundred and twenty-six blood and 310 urine samples were analysed during surveillance. Thirty-two patients had CMV infection (50%), 12 of whim progressed to HCMV disease. Detection of HCMV in either blood or urine was significantly associated with the presence or development of HCMV disease (blood, $P < 0.00001$; urine, $P = 0.0033$). All cases of HCMV disease were detected as PCR-positive in blood, although due to sampling only 50% of these patients were PCR-positive prior to disease onset. HCMV infection and disease were more likely in patients who suffered rejection ($P < 0.001$). In addition, the median amounts of augmented prednisolone were higher in patients with HCMV infection and disease. In all cases, augmented prednisolone preceded HCMV infection/disease. There was no statistical association between CMV infection and death. Overall, the results show that routine use of PCR for HCMV in surveillance samples of blood and urine of liver transplant recipients can provide diagnostic and prognostic information. However, its ability to provide prognostic information is directly related to the availability of appropriate surveillance samples, emphasising the importance of the routine acquisition of such samples in patient management to allow preemptive anti-HCMV therapy. ***J. Med. Virol. 51:152–158, 1997.***

KEY WORDS: glycoprotein b; antiviral therapy; ganciclovir

INTRODUCTION

Human cytomegalovirus (HCMV) is responsible for severe and often life-threatening disease in immunocompromised individuals [Griffiths, 1994]. Disease manifestations include pneumonitis, gastrointestinal tract lesions, retinitis, and hepatitis. In transplant recipients, HCMV infection may be due to reactivation of latent virus, or it may be acquired exogenously, either as a primary infection or as a reinfection. Various risk factors for HCMV infection and disease have been identified in different transplant groups. In the liver transplant recipient, the large volume of blood products required in the peritransplant period represents a further risk factor in addition to transplantation of a seropositive organ into a seronegative recipient and the use of antithymocyte immunoglobulin [Dummer et al., 1985; Barkholt et al., 1990; Fox et al., 1988; Gorensek et al., 1990; Paya et al., 1989; Singh et al., 1988]. We and others have previously shown that HCMV disease can be predicted by the detection of HCMV viraemia and viruria in surveillance cultures undertaken in bone marrow [Webster et al., 1993], liver [Pillay et al., 1992], and renal [Pillay et al., 1993] transplant recipients. The advent of more rapid and potentially more sensitive methods to detect HCMV, such as the polymerase chain reaction (PCR) and the antigenaemia test, has enabled many groups to show that both methods can yield diagnostic [Kidd et al., 1993; van Dorp et al., 1992; Einsele et al., 1991a] and in some cases prognostic [van-der-Bij et al., 1988] information. Since the risk period for HCMV disease is usually the first 3 months posttransplant, the regular screening of transplant patients for the presence of HCMV in blood and other specimens is of the utmost importance in effective patient management through appropriate antiviral intervention.

Previously, we reported the use of PCR for HCMV in

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TABLE I. Relationship Between Detection of HCMV in Blood or Urine and Development of HCMV Disease Following 76 Transplantations

	HCMV disease			
PCR result	Yes	No	Totals	*P* value
Blood +	12	13	25	
Blood –	0	51	51	<0.00001
Totals	12	64	76	
Urine +	7	7	14	
Urine –	6	56	62	=0.0033
Totals	13	63	76	

TABLE II. Median Number of Clinical Samples Collected From Patients With and Without HCMV Disease

	HCMV disease		
Sample	Yes	No	*P* value[a]
Blood	8	3	=0.005
Urine	7	2.5	=0.003
Totals (blood + urine)	14	4.5	<0.0002

[a]Mann-Whitney test.

a prospective study of renal, bone marrow, and liver patients and correlated the results with those of conventional cell culture and detection of early antigen fluorescent foci (DEAFF) testing [Kidd et al., 1993]. The results demonstrated that PCR offered prognostic advantages above the other methods for the detection of future disease due to HCMV in all patient groups. However, the number of liver transplant recipients analysed in this study was relatively small. At present, there is a paucity of data regarding the use of carrying out PCR for HCMV in blood and urine in the routine surveillance of liver transplant recipients. Indeed, one study has claimed that PCR is significantly worse than cell culture at providing prognostic information on HCMV disease in the liver transplant recipient [Delgado et al., 1992]. More recently, however, PCR of serum for the detection of HCMV has been shown to be the best predictor of the development of symptomatic HCMV infection in liver transplant recipients [Patel et al., 1995].

In order to provide further insight into the diagnostic and prognostic use of PCR for the detection of HCMV in blood DNA extracts and urine, a clinicopathological audit was undertaken of all liver transplant recipients transplanted during the period January 1993 to June 1994 at this institution. Such a study has been possible because PCR has been our method of choice since January 1993 for carrying out routine rapid surveillance of blood and urine for HCMV. This study provides the opportunity to compare PCR to previous results with cell culture from our laboratory and to indicate whether PCR is the most appropriate method for the rapid detection of HCMV in routine surveillance samples in the liver transplant recipient.

MATERIALS AND METHODS

Patients

Sixty-four consecutive liver transplant patients who received a total of 76 transplants (11 patients had two transplants and one patient had three transplants) were studied. Indications for liver transplantation were fulminant liver failure (n = 2), alcoholic cirrhosis (n = 9), cryptogenic cirrhosis (n = 5), primary biliary cirrhosis (n = 7), primary sclerosing cholangitis (n = 12), primary sclerosing cholangitis/cholangiocarcinoma (n = 2), hepatitis C cirrhosis (n = 20), hepatitis C/alcoholic cirrhosis (n = 1), hepatitis B/D cirrhosis (n = 1), Budd-Chiari syndrome (n = 2), amyloidosis (n = 1), and late-onset hepatitis (n = 1).

Patients were followed up weekly for the first month following discharge, then at 3-weekly intervals for the next 2 months, and then 3-monthly. Protocol liver biopsies were performed on days 5, 10, 15, and 25 post-transplant and whenever indicated clinically. Biopsies were carried out using a transjugular route or a plugged technique if coagulation parameters were outside those considered safe for a standard liver biopsy. The first transplant was undertaken in January 1993, the last transplant in June 1994. In the present paper, follow-up has occurred to 1 October 1994 (median follow-up, 6 weeks; range, 2 days–11 months). Forty patients remained alive at this time. The causes of death in the remaining 24 patients were pneumonia (n = 6), cerebral haemorrhage (n = 5), fulminant liver failure (n = 1), renal failure (n = 1), myocardial infarction (n = 1), renal sepsis (n = 2), adult respiratory distress syndrome (n = 2), nonfunctional graft (n = 3), cardiac failure (n = 1), hepatic infarction (n = 1), and CMV pneumonitis (n = 1).

Immunosuppressive Regimens

Standard immunosuppression consisted of methylprednisolone (0.8 mg/kg) intravenously (i.v.), followed by prednisolone (1 mg/kg) orally together with azathioprine (1.5 mg/kg) and cyclosporin (initial dose 4 mg/kg/day i.v. or 10 mg/kg/day orally), adjusted according to plasma concentration and renal function. Azathioprine and cyclosporin were continued long-term, with reduction of steroid over 4–6 weeks. No patient had antithyroglobulin (ATG) for induction immunosuppression. Rejection episodes were treated routinely by i.v. methyl prednisolone, 1 g daily for 3 days, for a maximum of two courses. If rejection was not controlled, OKT3 for 5 days was added, and if OKT3 had been used, ATG was used. Only three patients were prescribed ATG in this study population for periods of 1, 5, and 7 days, respectively.

Prophylaxis and Treatment Regiments

All patients received acyclovir 5 mg/kg tds i.v. for prophylaxis of herpes simplex infection, followed by acyclovir 200 mg qid orally to at least 1 month post-transplant. In addition, all patients received ampicillin 1 g qid, netilmicin 3.5 mg/kg bd, and metronidazole 500 mg tds i.v. for 2–3 days postoperatively as microbial

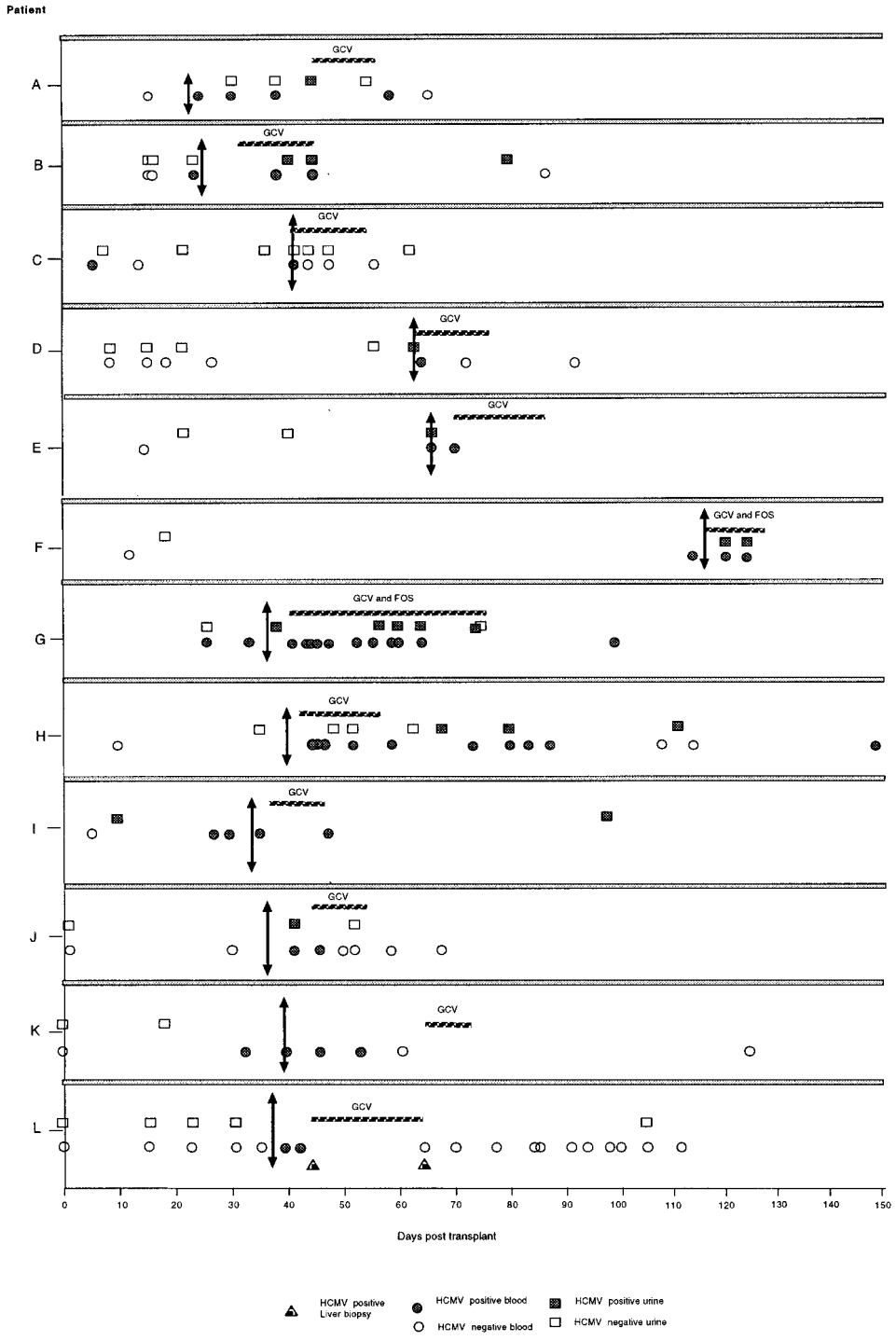

Fig. 1. Temporal association of HCMV in blood and urine in the 12 patients who suffered HCMV disease posttransplant. Onset of disease is shown as a vertical doubled-headed arrow. Duration and type of therapy is shown as a horizontal shaded bar. In addition, liver biopsy samples that were positive for HCMV by culture or histopathology are noted. The disease manifestation in each patient (pt) was as follows: pt A, pyrexia; pt B, oscillatory pyrexia; pt C, HCMV pneumonia; pt D, shortness of breath, HCMV pneumonitis; pt E, pyrexia, probable HCMV pneumonia; pt F, pyrexia, shortness of breath, HCMV pneumonitis resulting in death; pt G, extensive period of pyrexia, abdominal pain; pt H, prolonged pyrexia, raised liver function tests, HCMV hepatitis; pt I, pyrexia, rectal pain; pt J, pyrexia with diarrhoea; pt K, alveolar shadow, shortness of breath; pt L, oscillatory pyrexia, HCMV hepatitis.

prophylaxis. Amphotericin suspension (5 ml qds) was given for fungal prophylaxis.

For patients in whom HCMV disease was diagnosed, immunosuppression was reduced and therapy with i.v. ganciclovir (5 mg/kg bd) initiated, with dosage adjustment according to renal function. Human immune globulin was given i.v. in conjunction with ganciclovir in cases of HCMV pneumonitis. If the HCMV disease failed to respond to ganciclovir therapy, foscarnet (60 mg/kg q8 or 90 mg/kg q12) was given instead. Dosing was adjusted according to patients' creatinine clearance. In addition to virological screening, regular bacterial screening was undertaken. Intravenous cefotaxime 2 g tds was given initially for infection until the results of bacterial isolation and sensitivity tests were available.

Virological Surveillance

Pretransplant HCMV and HSV antibody status from the recipient were assessed by in-house radioimmunoassays [Berry et al., 1986, 1987]. Donors from this centre were assessed in the same way, but, if from elsewhere, HCMV antibodies were measured by latex agglutination.

Samples of urine and throat swabs in viral transport medium, 10 ml of blood in 500 IU preservative-free heparin, and 10 ml of clotted blood were collected weekly until discharge and then at regular out-patient appointments and when clinically indicated. Liver biopsy samples were sent routinely for histology and virology in viral transport medium.

"HCMV infection" was defined as the detection of HCMV by PCR, culture, DEAFF, or histology, "HCMV disease" was defined as HCMV infection associated with symptoms characteristic of HCMV as follows:

1. Pyrexia with viraemia: fever for at least 48 hr in the absence of rejection or bacterial/fungal infection.
2. Hepatitis: Alteration in liver function tests in the absence of histological rejection or bacterial or fungal infection in association with HCMV detection in liver biopsy by virological and/or histological techniques.
3. Pneumonitis: chest symptoms and/or characteristic chest radiograph pattern, unresponsive to antibiotics and with evidence of HCMV infection in bronchoalveolar lavage fluid.

PCR

PCR was used to detect the HCMV genome in urine and blood samples using primers specific for a 149 base pair fragment of the HCMV glycoprotein B gene [Darlington et al., 1991]. DNA was extracted from whole blood (0.2 ml) using commercially available extraction columns (QIAamp protocol; Qiagen, Chatsworth, CA), while urine samples were tested directly. Five microlitres of sample were diluted 1/20 in a reaction mix, giving a working composition of 25 mM Tris (pH 8.4), 17 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 10 mM β-mercaptoethanol, 0.01% gelatin, 200 μM dNTPs, 0.2 μM primers, and 10 U/ml Taq polymerase in aqueous solution. A further dilution was performed so that each sample was also tested at a 1/400 dilution to overcome possible problems due to the presence of inhibitory substances in the specimens. Assay sensitivity was assessed to be 10 copies. Appropriate controls were included as described in Kidd et al. [1993] and precautions against contamination taken according to the recommendations of Kwok and Higuchi [1989]. Thermal cycling was performed using a Perkin-Elmer Cetus (Norwalk, CT) GeneAmp PCR System 9600, with an initial denaturation for 4 min at 94°C followed by 40 cycles of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C. PCR products were electrophoresed through a 3% w/v agarose gel containing 0.5 μg/ml ethidium bromide in TBE. Bands were observed using a UV transilluminator. A specific band obtained with either or both sample dilutions was interpreted as "reactive," and the sample was repeat tested. Two reactive results were required before a specimen was reported as positive.

Statistical Analysis

Distributions of values between groups were compared using the χ^2 test or, where appropriate, Fisher's exact test. Differences between medians of groups were assessed by the Mann-Whitney U test.

RESULTS

HCMV Detection by PCR and Its Relationship to HCMV Disease

Of the 64 patients who received 76 organs, 40 (62.5%) remain alive. A total of 626 blood samples and 310 urine samples were obtained from these 64 liver transplant patients. The median numbers of blood and urine samples for each patient were seven (range 1–48) and four (range 0–30), respectively. The HCMV detection rate by PCR in blood was 27% and in urine 10.6%. Thirty-two recipients had HCMV infection, of whom 12 had HCMV disease (18.75%). The correlation between the presence of HCMV DNA in urine or blood and the disease status of the recipients is shown in Table I. HCMV presence in both urine and blood by PCR was significantly associated with appearance/presence of HCMV disease. All 12 cases of disease were PCR-positive for HCMV in the blood, although PCR positivity preceded disease in only six of these patients. In contrast, 54% of the same patients were positive in urine.

There was a significant difference in the median number of samples from patients with HCMV disease compared to those without disease, indicating that samples were being collected preferentially from ill patients, in addition to true surveillance samples, and that there was an increased probability of detecting HCMV disease with frequent sampling (Table II). Analysis of the timing of sample collection from the 12 patients with HCMV disease is shown in Figure 1. In all 12 patients, HCMV was detected in the urine or blood within 15 days of disease onset. In some cases—for example, patients B, G, K, and I—HCMV was detected in the blood prior to clinically overt disease.

TABLE III. Association of Rejection With HCMV Infection and Disease

	HCMV disease*		HCMV infection**	
Rejection	Yes	No	Yes	No
Yes	12	42	28	27
No	0	22	3	18
Totals	12	64	31	45

*$P = 0.022$. **$P = 0.006$.

However, in other instances (patients E, H, and J), HCMV was detected in the blood following disease onset. It should be noted that the ability to detect HCMV in the blood prior to the onset of HCMV disease was significantly correlated with the availability of surveillance samples in the preceding 20 days. In patient F, no surveillance samples were obtained between day 18 and day 106 due to patient relocation to another institution. This patient suffered HCMV disease and was treated with a course of ganciclovir followed by foscarnet. The mean time between the first detection of HCMV in the blood and the detection of HCMV disease was -4 ± 9.7 days.

Five of the 12 patients with HCMV disease (42%) died compared to 19 of the remaining 52 liver recipients (36%, P = not significant). Likewise, there was no statistical association between HCMV infection and death.

Association of HCMV Infection With Rejection

Fifty-seven recipients had rejection episodes and received augmented prednisolone. Three received ATG in addition, and 12 received OKT3, 15 receiving either ATG or OKT3. The correlation between HCMV infection and disease and the incidence of rejection is shown in Table III. The data show that there was a significant relationship between rejection and both CMV disease and infection. In all cases, augmented prednisolone treatment for rejection preceded the temporal appearance of HCMV infection and disease. Analysis of the total augmented quantity of prednisolone (in grams) with respect to HCMV infection or disease revealed that both were more common in patients receiving higher doses of augmented prednisolone (HCMV infection $P = 0.0001$, CMV disease $P = 0.003$). The distribution of augmented prednisolone in these groups is shown graphically in Figure 2A and B. Patients receiving OKT3 were more likely to have HCMV infection (9/12 vs. 22–64, $P = 0.02$), but there was no significant difference between HCMV disease incidence in the OKT3-treated or nontreated patients.

HCMV Infection/Disease and Blood Products

There was no difference between either administration of blood products or the median levels of blood products administered between patients who suffered HCMV infection or disease and those who remained HCMV infection/disease-free.

HCMV Infection and Disease in Patient Subgroups

The prevalence of HCMV infection and disease in patients stratified according to donor and recipient serological status for HCMV is shown in Table IV. Disease was most frequent in patients with primary infection (i.e., donor infection or acquired through other sources). However, there were no statistically significant differences between any of the combinations with respect to incidence of HCMV infection or disease.

DISCUSSION

The results of the present study demonstrate that routine surveillance of liver transplant recipients for HCMV using PCR provides both diagnostic and prognostic information. Such data are important for patient management and allow preemptive therapy with anti-HCMV drugs in patients who have a high risk of developing HCMV disease. The overall infection rate detected in this study (50%) is higher than that reported in our previous studies using conventional cell culture (37%, P = not significant) [Pillay et al., 1992]. In this context, the sensitivity of the PCR system used in this study was 10 genome equivalents, which equates to 400 genomes/ml blood. It is possible that nested PCR procedures would improve this level of sensitivity but may also detect more patients with latent or low level HCMV infection who are not destined to develop HCMV disease. HCMV was detected in the blood and/or urine of all the patients who experienced HCMV disease and was associated with a high negative predictive value. The timing of detection of HCMV in the blood and urine of patients with HCMV disease revealed that, when samples were available, HCMV can be detected prior to the onset of disease. Unfortunately, in the case of some patients, such routine surveillance samples were not dispatched to the laboratory and, hence, HCMV was detected either at the onset of disease or, in some cases, following disease onset. Thus, it was possible to show a significant association between the number of samples collected and HCMV disease. Clearly, on the basis of results generated in this and other studies [Pillay et al., 1992; Patel et al., 1995; Schmidt et al., 1993], the value of surveillance samples in the identification of patients most at risk of developing HCMV disease should stimulate physicians to be more rigorous in taking such specimens, especially in the first 8 weeks posttransplantation. Whilst this may present practical problems due to the current pressure to get patients out of hospital, often to distant referral sites, as PCR does not require viable virus, it could potentially be used on samples of blood and urine mailed regularly to the transplant centre.

In the patients analysed here, a major risk factor for an increased probability of HCMV infection and disease was the augmented use of prednisolone, which correlated with the appearance of rejection episodes in these patients. It is possible that HCMV infection is a manifestation of the rejection, necessitating increased

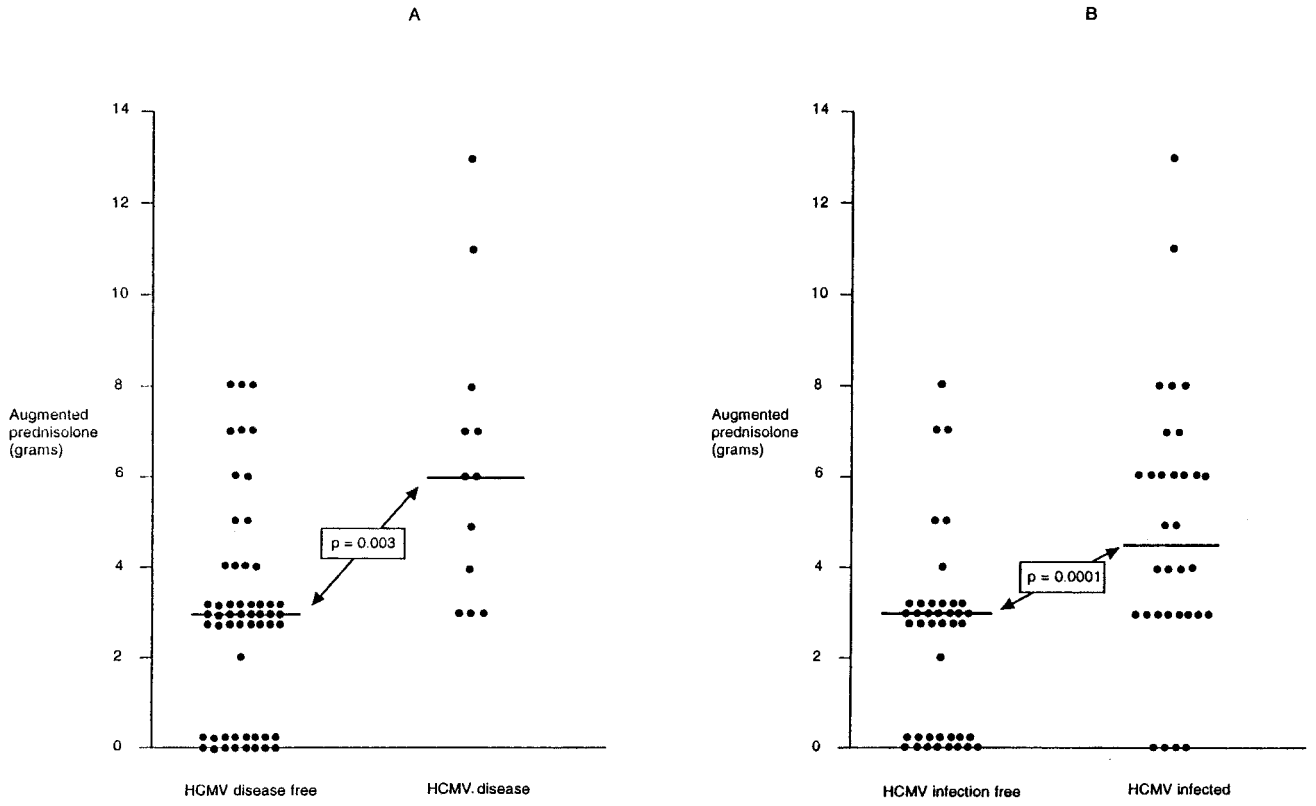

Fig. 2. **A:** Comparison of the total amount of augmented prednisolone (in grams) administered in patients who suffered HCMV disease or who remained HCMV disease-free. Each solid circle represents a separate individual. **B:** Comparison of the total amount of augmented prednisolone (in grams) administered in patients who suffered HCMV infection or who remained HCMV infection-free. Each solid circle represents a separate individual.

TABLE IV. Occurrence of HCMV Infection and HCMV Disease in Four Patient Subgroups Defined by Donor and Recipient Serostatus at the Time of Transplant*

HCMV	No. recipients	Infection	Disease
R + D +	29	8	3
R + D −	18	8	1
R − D +	15	9	6
R − D −	10	4	2
Totals	72[a]	29	12

*There was no significant difference in incidence of HCMV infection disease and the proportion of patients with infection suffering disease between the R–D + and R–D − groups.
[a]Four recipients with unknown donor status.

immunosuppression, or that HCMV is the cause of the rejection [O'Grady et al., 1988]. It is difficult to address this issue directly, but the finding that rejection preceded HCMV infection in all cases argues in favor of the former possibility, which would be consistent with results from cardiac and renal transplant recipients [Velasco et al., 1984; Gorensek et al., 1988]. OKT3 was used in 17% of patients and was associated with an increased occurrence of HCMV infection but not disease. In contrast, a recent study has shown that use of OKT3 in liver transplant recipients leads to an increase in both HCMV infection and disease [Portela et al., 1995]. However, these studies were carried out using cell culture for HCMV detection rather than PCR and more prolonged use of OKT3 (10 days' therapy) than was used in the treatment of patients in the present study (5 days' therapy). Interestingly, there was no significant association between increased blood product usage and HCMV infection or disease. Although this finding is not in agreement with our previously published data [Pillay et al., 1992], it should be noted that our earlier study used cell culture methods for HCMV detection. In addition, improved surgical procedures over the years have resulted in a reduced amount of blood product usage.

As expected, the likelihood of HCMV infection was greatest in seronegative recipients of a seropositive donor organ, although the numbers of patients analysed precluded statistically significant differences between incidence of disease and infection according to other combinations of donor and recipient serostatus for HCMV. There was no correlation between HCMV infection or disease and death. It should be noted that all cases of HCMV disease were treated promptly at diagnosis. However, it can be seen from the longitudinal analysis of patients with HCMV disease that preemptive therapy was not used in all cases and partly reflects the availability of samples in the period preceding disease onset.

The efficacy of treatment with ganciclovir can be assessed on a clinical basis (resolution of disease) and by

laboratory parameters (for example, cessation of DNAemia) [Einsele et al., 1991b]. In order for the latter to be effective, frequent samples must be obtained throughout the period of therapy. It is likely that fully quantitative measures of HCMV load will be the most informative parameter in studies of HCMV pathogenesis and responses to antiviral chemotherapy. In this context, we provided recent evidence for the central role of HCMV load in HCMV pathogenesis in renal transplant recipients [Fox et al., 1995] and are currently investigating whether the same is true in liver transplant recipients.

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REFERENCES

Barkholt LM, Ericzon B-G, Forsgren M, Andersson JP (1990): Cytomegalovirus infections in liver transplant patients: Incidence and outcome. Transplantation Proceedings 22:235.

Berry NJ, Grundy JE, Griffiths PD (1986): An improved radioimmunoassay method for the detection of IgG antibodies against cytomegalovirus. Journal of Virological Methods 13:343.

Berry NJ, Grundy JE, Griffiths PD (1987): Radioimmunoassay for the detection of IgG antibodies to herpes simplex virus and its use as a prognostic indicator of HSV excretion in transplant recipients. Journal of Medical Velocity 21:147.

Darlington J, Super M, Patel K, Grundy JE, Griffiths PD, Emery VC (1991): Use of the polymerase chain reaction to analyse sequence variation within a major neutralising epitope of glycoprotein B (gp58) in clinical isolates of human cytomegalovirus. Journal of General Virology 72:1985.

Delgado R, Lumbreras C, Alba C, et al. (1992): Low predictive value of polymerase chain reaction for diagnosis of cytomegalovirus disease in liver transplant recipients. Journal of Clinical Microbiology 30:1876.

Dummer JS, White LT, Ho M, Griffith BP, Hardesty RL, Bahson HT (1985): Morbidity of cytomegalovirus infection in recipients of heart or heart-lung transplants who receive cyclosporine. Journal of Infectious Diseases 152:1182.

Einsele H, Ehninger G, Steidle M, et al. (1991a): Polymerase chain reaction to evaluate antiviral therapy for cytomegalovirus disease. Lancet 338:1170.

Einsele H, Steidle M, Vallbracht A, Saal JG, Ehninger G, Muller C (1991b): Early occurrence of human cytomegalovirus infection after bone marrow transplantation as demonstrated by the polymerase chain reaction. Blood 77:1104.

Fox AS, Tolpin MD, Baker AL, et al. (1988): Seropositivity in liver transplant recipients as a predictor of cytomegalovirus disease. Journal of Infectious Disease 157:383.

Fox JC, Kidd IM, Griffiths PD, Sweny P, Emery VC (1995): Longitudinal analysis of cytomegalovirus load in renal transplant recipients using a quantitative polymerase chain reaction: Correlation with disease. Journal of General Virology 76:309.

Gorensek MJ, Carey WD, Vogt D, Goormastic M (1990): A multivariate analysis of risk factors for cytomegalovirus infection in liver transplant recipients. Gastroenterology 98:1326.

Gorensek MJ, Stewart RW, Keys TF, McHenry MC, Goormastic M (1988): A multivariate analysis of the risk of cytomegalovirus infection in heart transplant recipients. Journal of Infectious Diseases 157:515.

Griffiths PD (1994): Cytomegalovirus. In Zuckerman AJ, Banatvala JE, Pattison JR (eds): "Principles and Practice of Clinical Virology" Chichester: Wiley, pp 69.

Kidd IM, Fox JC, Pillay D, Charman H, Griffiths PD, Emery VC (1993): Provision of prognostic information in immunocompromised patients by routine application of the polymerase chain reaction for cytomegalovirus. Transplantation 56:867.

Kwok S, Higuchi R (1989): Avoiding false positives with PCR. Nature 339:237.

O'Grady JG, Alexander GHM, Sutherland S, et al. (1988): Cytomegalovirus infection and donor/recipient HLA antigens: Pathogenesis of vanishing bileduct syndrome after liver transplantation. Lancet 2:302.

Patel R, Smith TF, Epsey M, et al. (1995): A prospective comparison of molecular diagnostic techniques for the early detection of cytomegalovirus in liver transplant recipients. Journal of Infectious Diseases 171:1010.

Paya CV, Hermans PE, Wiesner RH, et al. (1989): Cytomegalovirus hepatitis in liver transplantation: Prospective analysis of 93 consecutive liver transplantations. Journal of Infectious Diseases 160:752.

Pillay D, Charman H, Burroughs AK, Smith M, Rolles K, Griffiths PD (1992): Surveillance for CMV infection in orthotopic liver transplant recipients. Transplantation 53:1261.

Pillay D, Ali AA, Liu SF, Kops E, Sweny P, Griffiths PD (1993): The prognostic significance of positive CMV cultures during surveillance of renal transplant recipients. Transplantation 56:103.

Portela D, Patel R, Larson-Keller JJ, et al. (1995): OKT3 treatment for allograft rejection is a risk factor for cytomegalovirus disease in liver transplantation. Journal of Infectious Diseases 171:1014.

Schmidt CA, Oettle H, Neuhaus P, et al. (1993): Demonstration of cytomegalovirus by polymerase chain reaction after liver transplantation. Transplantation 56:872.

Singh N, Dummer JS, Kusne S, et a. (1988): Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: Transmission by donated organ and the effect of OKT3 antibodies. Journal of Infectious Diseases 158:124.

van-der-Bij W, Schirm J, Torensma R, van-Son WJ, Tegzess AM, The TH (1988): Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. Journal of Clinical Microbiology 26:2531.

van Dorp WT, Vlieger A, Jiwa NM, et al. (1992): The polymerase chain reaction, a sensitive and rapid technique for detecting cytomegalovirus infection after renal transplantation. Transplantation 54: 661.

Velasco N, Catto GRD, Edward N, Engeset J, Moffat MAJ (1984): The effect of the dosage of steroids on the incidence of cytomegalovirus infections in renal transplant recipients. Journal of Infection 9:69.

Webster A, Blizzard B, Pillay D, Prentice HG, Pothecary K, Griffiths PD (1993): Value of routine surveillance cultures for detection of CMV pneumonitis following bone marrow transplantation. Bone Marrow Transplantation 12:477.